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Associations of cardiovascular and all-cause mortality events with oxygen uptake at ventilatory threshold

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Abbreviations

BMI = body mass index
CHD = coronary heart disease
CRF = cardiorespiratory fitness
CVD = cardiovascular disease
HDL-C = high-density lipoprotein cholesterol
HR = hazard ratio
IDI = integrated-discrimination-improvement
KIHD = Kuopio Ischaemic Heart Disease
NRI = net reclassification improvement
SCD = sudden cardiac death
SD = standard deviation
SBP = systolic blood pressure
VT = ventilatory threshold
VO₂ = oxygen uptake

ABSTRACT

Background: Oxygen uptake (VO_2) at ventilatory threshold (VT), is a cardiopulmonary exercise testing parameter which may be a proxy for peak VO_2 . We aimed to assess the associations of VO_2 at VT with sudden cardiac death (SCD), fatal coronary heart disease (CHD) and cardiovascular disease (CVD), and all-cause mortality.

Methods and Results: VO_2 at VT was assessed during a submaximal exercise test using respiratory gas analyzers in the Kuopio Ischemic Heart Disease cohort of 1,639 middle-aged men. Hazard ratios (HRs) (95% CIs) were assessed. During a median follow-up of 25.6 years, 121 SCDs, 202 fatal CHDs, 312 fatal CVDs, and 703 all-cause mortality events occurred. VO_2 at VT was correlated with peak VO_2 ($r = 0.90$) and linearly associated with each outcome. Comparing extreme quartiles of VO_2 at VT, the HRs (95% CIs) for SCD, fatal CHD, fatal CVD, and all-cause mortality on adjustment for established risk factors were 0.37 (0.18-0.78), 0.32 (0.18-0.57), 0.45 (0.30-0.69), and 0.50 (0.38-0.64) respectively. The HRs were 1.02 (0.36-2.91), 1.43 (0.63-3.25), 1.46 (0.79-2.71), and 1.02 (0.69-1.51) respectively on further adjustment for peak VO_2 . Addition of VO_2 at VT to a CVD mortality risk prediction model containing established risk factors significantly improved risk discrimination and reclassification at 25 years.

Conclusions: There are linear and inverse associations of VO_2 at VT with fatal cardiovascular and all-cause mortality events, which are dependent on peak VO_2 . Inclusion of VO_2 at VT in the standard established risk factors panel significantly improves the prediction and classification of long-term CVD mortality risk.

Keywords: Oxygen uptake at ventilatory threshold; peak oxygen uptake; cardiopulmonary exercise testing; risk prediction; fatal cardiovascular disease; all-cause mortality

1. Introduction

Peak oxygen uptake (VO_2) which is used as a measure of cardiorespiratory fitness (CRF), is a cardiopulmonary exercise testing (CPX) parameter which is considered to be one of the best measures for assessing cardiovascular fitness and aerobic capacity.[1] Peak VO_2 has been consistently shown to be inversely and independently associated with incident cardiovascular disease (CVD) events, cardiovascular mortality, and total mortality in large-scale epidemiological cohorts.[2-4] Evidence also suggests that Peak VO_2 adds additional prognostic value beyond established risk factors in predicting vascular disease and mortality risk.[2, 5, 6] There is a growing appreciation of the value of exercise testing at the anaerobic threshold, a concept which was introduced by Wasserman and colleagues.[7] Oxygen uptake at ventilatory threshold (VT), often referred to as the anaerobic threshold, is a parameter assessed at submaximal level of CPX.[8] For majority of healthy individuals, the anaerobic threshold lies at exercise intensities between 50% and 75% of VO_2 max; while in trained endurance athletes, it can reach intensities as high as 80% of VO_2 max.[9] Beyond the anaerobic threshold of exercise testing, there is accumulation of lactic acid which eventually results in metabolic acidosis.[7] Unlike peak VO_2 which is restricted by the capability of the cardiorespiratory system to deliver oxygen to exercising muscles and thus may not be the best measure of endurance, VO_2 at VT is highly correlated with endurance performance.[10, 11] It has been suggested that the anaerobic threshold could be a determinant of physiological fitness and since it assesses the capacity to perform sustained aerobic activity, VO_2 at VT is considered a reliable index of aerobic capacity. Though there is emerging evidence on the potential clinical application of VO_2 at VT as a CPX variable, there are still persisting uncertainties. In 2016, the European Association for Cardiovascular Prevention & Rehabilitation and the American Heart Association (AHA) updated their clinical recommendations for CPX data assessment in specific patient populations; and recommended the inclusion of VO_2 at VT in the algorithm for presurgical assessment.[12] However, the clinical value of VO_2 at VT in general populations has not been established and there is no prospective data (to our knowledge) on the nature of the relationships of VO_2 at VT with

adverse outcomes. Given the relative ease at which this submaximal exercise testing measure can be assessed noninvasively using respiratory gases, it will be clinically useful to know if VO_2 at VT can be used as a suitable proxy for peak VO_2 .

Our first objective to assess the nature and magnitude of the associations of VO_2 at VT with the risk of sudden cardiac death (SCD), fatal coronary heart disease (CHD) and CVD events, and all-cause mortality using the established Kuopio Ischemic Heart Disease (KIHD) population-based cohort comprising healthy middle-aged men from eastern Finland. A second objective was to investigate the extent to which VO_2 at VT measurements could improve the prediction of CVD mortality when added to conventional risk factors.

2. Materials and Methods

2.1. Study population

The KIHD risk factor study is a prospective epidemiologic study of the incidence of atherosclerotic CVD and other related chronic disease outcomes among Finnish adults ($N=3,433$). Participants of the KIHD study were selected to be a representative sample of men aged 42-61 years living in the city of Kuopio and its surrounding rural communities in eastern Finland. A complete description of the study design, objectives, and sampling strategy has been previously described.[13] Briefly, of the 3,433 randomly selected to be potentially eligible, 3235 were found to be eligible for inclusion into the study and of this number, 2,682 (82.9%) volunteered to participate in the study. Baseline examinations were performed between March 1984 and December 1989. Of the 2,682 men, 1,027 had missing measurements on CPX parameters, relevant covariates, and our specified outcomes. Of these, 694 men did not have data on VO_2 at VT measurements because they could not participate in exercise testing measurements as a result of prevalent disease including musculoskeletal problems or could not exercise to a sufficient intensity level to reach VO_2 at VT. The final cohort comprised of 1,639 men with complete information with VO_2 at VT and peak VO_2 measurements, relevant risk markers, and the main outcomes. Less than

1% of data was missing for most covariates; whereas missing data was 25.9% for VO_2 at VT and 11.7% for peak VO_2 as a result of those participants unable to undergo exercise protocol because of musculoskeletal and orthopedic problems. The research protocol and study design were approved the institutional review board of the University of Eastern Finland, Kuopio, Finland and all study participants provided written informed consent.

2.2. Assessment of VO_2 at VT

Oxygen uptake was assessed using a respiratory gas exchange analyzer (Medical Graphics, MCG, St. Paul, Minnesota) during a submaximal symptom-limited cycle ergometer exercise tolerance test performed between 8:00 am and 10:00 am.[14] The standardized testing protocol comprised a graded increase in the workload of 20 W/min until exhaustion. The gas analyzer expressed VO_2 as the average values recorded over 8 seconds. VO_2 at VT was defined based on changes in respiratory gases (VO_2 and VCO_2) during exercise test. In this study, VO_2 at VT was defined at the level of exercise tests at which an increase in the ventilatory equivalent for oxygen (VE/VO_2) occurs without an increment in the ventilatory equivalent for carbon dioxide production (VE/VCO_2). Peak VO_2 which was assessed using a respiratory gas exchange analyzer during the cycle ergometer exercise test was used as a measure of CRF, as described in previous reports.[4, 14] To ensure safety, all tests were supervised by an experienced physician with the assistance of an experienced nurse.

2.3. Assessment of covariates

Information on covariates was obtained by history, examinations, and measurements during the baseline visit. Detailed description of blood sample collection, physical measurements, assessment of lifestyle characteristics, and assays for lipids, lipoproteins and biochemical factors have been provided in previous reports.[15, 16] Serum levels of triglycerides were assessed enzymatically (Boehringer Mannheim, Mannheim, Germany). Fasting plasma glucose was measured by the glucose dehydrogenase

method (Merck, Darmstadt, Germany) after protein precipitation by trichloroacetic acid. Smoking, use of antihypertensive and lipid-lowering medications, and medical history were assessed by standardized self-administered questionnaires.[15] Alcohol consumption was assessed using the Nordic Alcohol Consumption Inventory. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mm Hg, diastolic blood pressure (DBP) ≥ 90 mm Hg, prior physician diagnosis of hypertension, or use of antihypertensive medications. History of CHD was based on a previous myocardial infarction, angina pectoris, the use of nitroglycerin for chest pain once a week or more frequently or chest pain. Physical activity was assessed from a 12-month physical activity history modified from the Minnesota Leisure-Time Physical Activity Questionnaire,[17] described in detail previously.[14] Adulthood socioeconomic status (SES) was assessed as a combined measure of income, education, occupation, occupational prestige, material standard of living, and housing conditions.[18] Heart rate was recorded at rest.

2.4. Ascertainment of outcomes

Our outcomes of interest were SCD, fatal CHD and CVD, and all-cause mortality events. The ascertainment procedures have been described previously. Deaths that occurred from study enrollment through to 2013 were included. In the KIID study, participants (using personal identification codes) are under continuous annual surveillance for mortality events as well as incident vascular events.[19] No losses to follow-up have been recorded in the KIID study. Both deaths and hospitalization events were ascertained from discharge lists, death certificates, hospital records, local hospitals, informant interviews, health practitioner questionnaires, study electrocardiograms, medico-legal reports, and vital statistics offices. The diagnostic classification of SCDs was based on symptoms, electrocardiographic findings, cardiac enzyme elevations, autopsy findings, and history of CHD together with the clinical history and findings from hospital and paramedic staff, details of which have been previously described.[4, 20, 21] CHD and CVD deaths were coded using the *International Classification of Diseases, Ninth Revision (ICD-9)*, and *International Statistical Classification of Diseases, 10th Revision (ICD-10)*, codes.

Documents were cross-checked in detail by two physicians. The Independent Events Committee, masked to clinical data, performed classification of outcomes.

2.5. Statistical analysis

We summarized baseline characteristics of study participants using descriptive statistics (i.e., means, medians, and percentages). Age-adjusted partial correlation coefficients were calculated to assess the cross-sectional associations of VO_2 at VT with risk markers. Cox proportional hazard models were used to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) for SCD, fatal CHD and CVD events, and all-cause mortality. The proportionality-hazards assumption was confirmed using Schoenfeld residuals.[22] We assessed the shape of the association between VO_2 at VT and each outcome, by calculating the HRs within quartiles of baseline VO_2 at VT and plotting these against the mean values of VO_2 at VT within each quartile. Floating variances were used as described previously.[23, 24] We modelled our exposure as both continuous [per unit (ml/kg/min) and 1 MET increase] and categorical (quartiles) variables. We calculated age-, multivariable-, and multivariable plus peak VO_2 -adjusted HRs for each outcome. The multivariable-adjusted models controlled for age, body mass index (BMI), SBP, high-density lipoprotein cholesterol (HDL-C), alcohol consumption, history of diabetes mellitus, smoking status, prevalent CHD, resting heart rate, physical activity, and SES. Subgroup analyses were performed using interaction tests to assess statistical evidence of effect modification by levels/categories of pre-specified individual level characteristics.

To assess whether adding information on VO_2 at VT to documented established risk factors is associated with improvement in prediction of CVD mortality risk, we calculated measures of discrimination for censored time-to-event data (Harrell's C-index [25]) and reclassification.[26, 27] To investigate the change in C-index on the addition of VO_2 at VT, two CVD mortality risk prediction models were fitted: one model based on traditional risk factors (i.e., age, SBP, history of diabetes, total cholesterol, HDL-C, and smoking) and the second model with these risk factors plus VO_2 at AT.

Reclassification analyses was restricted to the first 25 years and was assessed using the net-reclassification-improvement (NRI)[26, 27] and integrated-discrimination-improvement (IDI)[26] by comparing the model containing conventional risk factors to the predicted risk from the model containing conventional risk factors plus VO₂ at VT and secondly, the conventional risk factor model combined with peak VO₂. Reclassification analyses was based on predicted 25-year CVD mortality risk categories of low (<8%), intermediate (8 to <30%), and high (≥30%) risk as previously reported.[6] All statistical analyses were conducted using Stata version 14 (Stata Corp, College Station, Texas).

3. Results

3.1. Baseline characteristics and correlates of VO₂ at VT

The mean age at baseline was 52 [standard deviation (SD), 5] years. The baseline mean (SD) level of VO₂ at VT was 23.72 (6.10) ml/kg/min and the corresponding metabolic equivalent was 6.78 (1.74) (**Table 1**). There were weak to moderate inverse correlations of VO₂ at VT with age, physical measures (BMI, blood pressure, and resting heart rate), lipids (total cholesterol and triglycerides), and fasting plasma glucose. Positive correlations were observed for peak VO₂ (r = 0.90), HDL-C (r = 0.30) and physical activity (r = 0.11). Values of VO₂ at VT were lower by 96% in men with diabetes compared with men without diabetes, by 82% in current smokers compared with non-smokers, by 94% in men with a history of hypertension compared with those without a history, and by 98% in men with a prevalent history of CHD compared with men without a history.

3.2. VO₂ at VT and outcome events

During a median (IQR) follow-up of 25.6 (20.1-27.1) years, a total of 121 SCDs, 202 fatal CHDs, 312 fatal CVDs, and 703 all-cause mortality events occurred. In analyses adjusted for several established risk factors (age, BMI, SBP, HDL-C, alcohol consumption, history of diabetes mellitus, smoking status, prevalent CHD, resting heart rate, physical activity, and SES), VO₂ at VT showed graded and inverse

associations with SCD, fatal CHD, fatal CVD, and all-cause mortality (**Figure 1**). **Table 2** summarizes the associations of VO₂ at VT with each outcome assessed. The age-adjusted HRs (95% CIs) per unit increase in VO₂ at VT for SCD, fatal CHD, fatal CVD, and all-cause mortality were 0.90 (0.87-0.93), 0.89 (0.87-0.92), 0.92 (0.90-0.94), and 0.94 (0.93-0.95) respectively. The HRs (95% CIs) were only minimally attenuated to 0.94 (0.90-0.98), 0.93 (0.90-0.96), 0.94 (0.92-0.97), and 0.95 (0.94-0.97) respectively after multivariable adjustment. Further adjustment for peak VO₂ attenuated the associations. Alternatively, comparing the top versus bottom quartile of VO₂ at VT, the age-adjusted HRs (95% CIs) for SCD, fatal CHD, fatal CVD, and all-cause mortality were 0.18 (0.09-0.37), 0.18 (0.10-0.31), 0.27 (0.19-0.40), and 0.39 (0.31-0.49) respectively. After multivariable adjustment, the corresponding hazard ratios were 0.37 (0.18-0.78), 0.32 (0.18-0.57), 0.45 (0.30-0.69), and 0.50 (0.38-0.64) respectively. The associations were less robust on further adjustment for peak VO₂ (**Table 2**). The associations did not generally vary significantly by levels or categories of several clinically relevant characteristics, except for some evidence of effect modification by SBP for all outcomes. There was some evidence of effect modification by history of CHD for fatal CVD and all-cause mortality outcomes (*P* for interaction < 0.05). Though the associations were in the same direction, stronger protective associations were observed in men with a prevalent history of CHD compared with men without a prevalent history of CHD (**Appendix Supplements 1 and 2**). In a sensitivity analysis which was limited to a sample of 1,620 men after 19 men with atrial fibrillation were excluded, the associations remained consistent. Direct comparisons were made to the associations of VO₂ at AT with outcomes using peak VO₂ in the same set of participants. Peak VO₂ was inversely and independently associated with each outcome. The associations were also independent of VO₂ at VT (**Appendix Supplement 3**).

3.3. VO₂ at VT and CVD mortality risk prediction

A CVD mortality risk prediction model containing established risk factors yielded a C-index of 0.7023 (95% CI: 0.6741-0.7305). After addition of information on VO₂ at VT, the C-index was 0.7249 (0.6973-

0.7525), representing a significant increase of 0.0226 (0.0070 to 0.03822; $P=0.004$). After taking into account inappropriate reclassification, there was a significant improvement in the classification of participants into predicted 25-year CVD mortality risk categories (NRI: 8.02%, 2.66-13.38%; $P=0.003$). The IDI was 0.0280 (0.0191-0.0369; $P<0.001$).

To compare the predictive ability of VO_2 at VT with peak VO_2 in the same participants, information on peak VO_2 was added to the model containing conventional risk factors. There was a C-index change of 0.0324 (95% CI: 0.0140-0.0508; $P = 0.001$), after adding VO_2 peak in the model. There was a significant improvement in the classification of participants into CVD mortality risk categories (NRI: 10.44%, 4.46-16.43%; $P=0.001$). The IDI was 0.0406 (0.0292-0.0520; $P<0.001$).

4. Discussion

In this large-scale population-based prospective cohort study of apparently healthy Finnish men aged at 42-61 at inclusion and with more than 25 years of follow-up, we found strong and linear associations of VO_2 at VT with cardiovascular and all-cause mortality events in analyses adjusted for several established cardiovascular risk factors. The associations were however partly dependent on peak VO_2 . Oxygen uptake at VT was observed to be modestly and inversely correlated with several established cardiovascular risk factors; of note was the strong positive correlation of VO_2 at VT with VO_2 . In further analysis that assessed the association of peak VO_2 with each outcome in the same set of participants, stronger inverse associations were demonstrated and which were independent of VO_2 at VT; which implies that peak VO_2 is a stronger risk indicator than VO_2 at VT for these outcomes. Though there was suggestion of evidence of effect modification by SBP and history of CHD, the associations remained generally consistent across several clinically relevant subgroups. The stronger protective effect of VO_2 at VT on fatal CVD and all-cause mortality events in men with a prevalent history of CHD may reflect previous findings which suggests that exercise training has more beneficial effects on adverse outcomes in individuals with pre-existing cardiometabolic disease compared with individuals without pre-existing

disease.[28, 29] Finally, addition of VO_2 at VT significantly improved measures of discrimination and reclassification for CVD mortality risk when added to established risk factors. Additional analyses in the same set of participants showed that the improvement provided by peak VO_2 assessment in the prediction of CVD mortality risk was somewhat better than that of VO_2 at AT.

4.1. Comparison with other studies

We are unable to compare the current findings with previous work, as no prospective study to date has evaluated the associations of VO_2 at VT with the risk of fatal outcomes. The results are however in line with findings from a recent study by our group using the KIHG cohort (unpublished data). We have shown that VO_2 at aerobic thresholds (a measure of exercise capacity and also assessed by submaximal level exercise testing) is protective of fatal cardiovascular and all-cause mortality events, which were consistent with graded relationships. In addition, our novel findings are not surprising, given that VO_2 at VT is a reliable measure of aerobic capacity.[8, 12] Peak VO_2 which is considered the gold standard measure of aerobic capacity [1] has been demonstrated to be inversely and independently associated with vascular risk and mortality outcomes in several large epidemiological prospective cohort studies.[2-4] Indeed, our findings also show that VO_2 at VT is strongly correlated with peak VO_2 . Our findings demonstrating VO_2 at VT to provide independent prognostic information for CVD mortality extend prior observations on the predictive ability of fitness on the risk of CVD mortality.[2, 5, 6]

4.2. Potential biological mechanisms

Compared with peak VO_2 , VO_2 at VT is more highly correlated with endurance[10, 11] and is a determinant of physiological fitness and therefore provides an index of sustained physical or aerobic activity. This therefore suggests that the biological mechanisms by which exercise training provides benefits on the cardiovascular system, may underlie the protective associations demonstrated in the current study. Physical activity exerts cardioprotective effects via (i) improvement in cardiovascular risk

factors such as blood pressure, biomarkers of insulin resistance, lipid and glucose levels, natriuretic peptides, and cardiac troponin T;[30-32] (ii) its anti-inflammatory actions, by reduction in levels of inflammatory markers such as IL-18 and C-reactive protein;[33, 34] (iii) regulation of white adipose tissue mass and adipokine expression;[35] (iv) improvement in endothelial function which ultimately slows the atherosclerotic cascade;[36, 37] (v) vagal control of heart rate and regulation of cardiac autonomic function;[38] and (vi) increase in cardiac output, left ventricular function, oxygen utilization, and the formation of collateral vessels.[36, 39] In a meta-analysis of 160 randomized controlled trials, Lin and colleagues demonstrated that exercise significantly improved CRF with beneficial modulation of several cardiometabolic markers.[40]

4.3. Implications of findings

The results of our study support a protective effect of VO_2 at VT on fatal cardiovascular and all-cause mortality outcomes, shows an incremental predictive value to CVD mortality of assessing VO_2 at VT, and also shows the ability of VO_2 at VT to reclassify subjects across clinically relevant thresholds of risk. The last decade has witnessed a growing volume of evidence in support of CPX, which has triggered the release of recommendations by several guideline bodies and associations.[8, 41] However, despite the numerous advances in the evidence base, the clinical application of CPX has not been well established, especially in general population settings. The European Association for Cardiovascular Prevention & Rehabilitation and the AHA have recently recommended VO_2 at VT to be used in presurgical risk assessment algorithms.[12] Because common physical activities do not require maximal effort, VO_2 at VT has also been used as an individualized exercise training prescription in both normal individuals and patients with cardiac conditions.[42, 43] Though the current findings suggest peak VO_2 as a stronger risk indicator and provides better improvement in the prediction of CVD mortality risk than VO_2 at VT, the overall data supports the consideration that VO_2 at VT as a CPX variable may be a suitable proxy for peak VO_2 or CRF in apparently healthy populations. Until recently, VO_2 at VT has not been utilized

widely because of technical difficulties in its measurement. However, with the introduction of respiratory gas analyzers and automated data processes, it is relatively easy to analyze and compute this index.[7] Unlike peak VO_2 which is determined during maximal or peak exercise testing and therefore may be difficult to assess during exercise limitations, during periods of low motivation, and in some patient populations;[44] VO_2 at VT can be safely assessed at submaximal exercise levels. For example, Boyne and colleagues observed in 59 patients with stroke that VO_2 at VT may be a more reliable measure of aerobic capacity than peak VO_2 . [45] It is acknowledged that it may not always be possible to determine VO_2 at VT reliably, especially in some patient populations, such as those with significant respiratory impairment or patients with heart failure.[7, 46] In addition, unlike VO_2 at VT, the assessment of peak VO_2 is limited by the ability of oxygen to be delivered to the exercising muscles and therefore peak VO_2 cannot be sustained for a long period.[47] Our findings are timely and shows VO_2 at VT to have potential applications in clinical settings. However, before any guideline recommendations can be made, further research is needed to replicate these findings in other populations and unequivocally establish the clinical utility of VO_2 at VT as a risk assessment tool for major adverse cardiovascular outcomes in the general population.

4.4. Strengths and limitations

We have reported the first prospective evaluation of the associations between VO_2 at VT and the risk of cardiovascular and all-cause mortality outcomes using a well characterised and established large population-based prospective cohort with a follow-up of over 25 years. The KIHd cohort had a high participation rate and there were no losses during follow-up which minimised the risk of selection bias. Our sample comprised a nationally representative ethnically and genetically homogeneous cohort which may reduce confounding by factors such as medical care. Participants in the KIHd cohort have been annually monitored and checked using established well-linked databases for outcome events, which were confirmed by physicians' review.[4, 5] Our analyses were comprehensive and employed adequate

statistical techniques, thereby ensuring the robustness of our results. We had access to and adjusted for several lifestyle and biological markers, assessed the shape of the relationships, conducted subgroup analyses which showed consistent direction of effect across several clinically relevant subgroups, and we have conducted risk prediction analyses. Some limitations should be considered when interpreting the results. The KIHD study included middle-aged Caucasian men from eastern Finland, so generalizability to other populations might be limited. However, we have no reason to believe that the pathophysiological pathways by which physical activity might have a protective effect on vascular and total mortality would be different in other age groups and populations of men. Our assessment of VO_2 at VT was based on ventilatory equivalents method which is an estimation compared to the gold standard V-slope method.[48] Though the V-slope method has significant advantages over the ventilatory equivalents method, a number of studies have reported that no significant differences in mean VO_2 at VT values between the two methods.[45, 48] We had no repeat measurements of VO_2 at VT, which could have underestimated the associations, especially given that reproducibility sub-studies of peak VO_2 in the KIHD study have reported high within-person variability (regression dilution ratio of 0.58) in peak VO_2 levels.[49] There was some missing data for our exposure variables which could have potentially led to loss of information. However, the missing data were for participants who could not undergo exercise testing because of musculoskeletal or orthopedic issues; and given that the exposures were assessed in relation to cardiovascular outcomes, our associations are unlikely to be affected by the missing data. Moreover, the magnitude of the associations and narrow confidence intervals of our estimates suggest there was adequate power to demonstrate these associations. Though we adjusted for a comprehensive panel of covariates, residual confounding remains a potential alternative explanation for our findings, due to the observational design of the study.

Conclusions

The results of this large prospective cohort study in middle-aged Caucasian men with long-term follow-up indicate strong linear and inverse associations of VO_2 at VT with fatal cardiovascular and all-cause mortality events, which are partly dependent on peak VO_2 . Additionally, VO_2 at VT significantly improves the prediction and reclassification of the long-term risk for CVD mortality beyond established risk factors.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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References

- [1] Noonan V, Dean E. Submaximal exercise testing: clinical application and interpretation. *Phys Ther.* 2000;80:782-807.
- [2] Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *Jama.* 2009;301:2024-35.
- [3] Hagnas MJ, Kurl S, Rauramaa R, Lakka TA, Makikallio TH, Savonen K, et al. The value of cardiorespiratory fitness and exercise-induced ST segment depression in predicting death from coronary heart disease. *Int J Cardiol.* 2015;196:31-3.
- [4] Laukkanen JA, Makikallio TH, Rauramaa R, Kiviniemi V, Ronkainen K, Kurl S. Cardiorespiratory fitness is related to the risk of sudden cardiac death: a population-based follow-up study. *J Am Coll Cardiol.* 2010;56:1476-83.
- [5] Laukkanen JA, Kurl S, Salonen R, Rauramaa R, Salonen JT. The predictive value of cardiorespiratory fitness for cardiovascular events in men with various risk profiles: a prospective population-based cohort study. *European Heart Journal.* 2004;25:1428-37.
- [6] Gupta S, Rohatgi A, Ayers CR, Willis BL, Haskell WL, Khera A, et al. Cardiorespiratory fitness and classification of risk of cardiovascular disease mortality. *Circulation.* 2011;123:1377-83.
- [7] Wasserman K, Whipp BJ, Koyl SN, Beaver WL. Anaerobic threshold and respiratory gas exchange during exercise. *J Appl Physiol.* 1973;35:236-43.
- [8] Balady GJ, Arena R, Sietsema K, Myers J, Coke L, Fletcher GF, et al. Clinician's Guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association. *Circulation.* 2010;122:191-225.
- [9] Joyner MJ, Coyle EF. Endurance exercise performance: the physiology of champions. *J Physiol.* 2008;586:35-44.
- [10] Kumagai S, Tanaka K, Matsuura Y, Matsuzaka A, Hirakoba K, Asano K. Relationships of the anaerobic threshold with the 5 km, 10 km, and 10 mile races. *Eur J Appl Physiol Occup Physiol.* 1982;49:13-23.
- [11] Farrell PA, Wilmore JH, Coyle EF, Billing JE, Costill DL. Plasma lactate accumulation and distance running performance. *Med Sci Sports.* 1979;11:338-44.
- [12] Guazzi M, Arena R, Halle M, Piepoli MF, Myers J, Lavie CJ. 2016 focused update: clinical recommendations for cardiopulmonary exercise testing data assessment in specific patient populations. *Circulation.* 2016;133:e694-711.
- [13] Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study. *Ann Clin Res.* 1988;20:46-50.

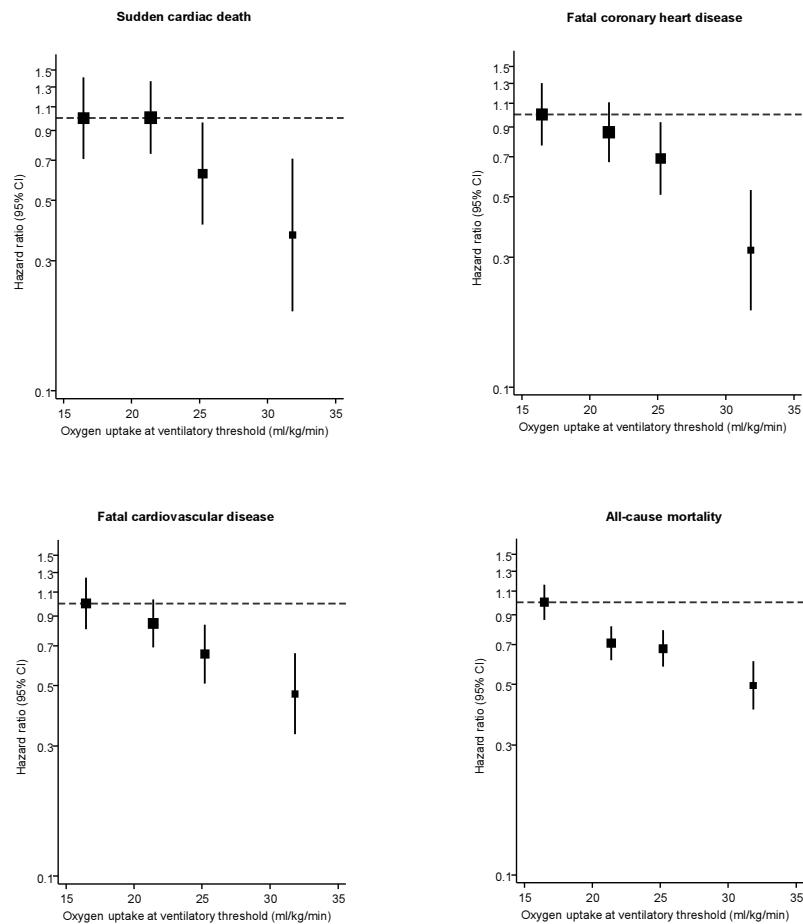
- [14] Lakka TA, Venalainen JM, Rauramaa R, Salonen R, Tuomilehto J, Salonen JT. Relation of leisure-time physical activity and cardiorespiratory fitness to the risk of acute myocardial infarction. *N Engl J Med*. 1994;330:1549-54.
- [15] Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation*. 1992;86:803-11.
- [16] Kunutsor SK, Khan H, Laukkanen JA. gamma-Glutamyltransferase and Risk of Sudden Cardiac Death in Middle-Aged Finnish Men: A New Prospective Cohort Study. *J Am Heart Assoc*. 2016;Feb 8;5(2). pii: e002858. doi: 10.1161/JAHA.115.002858.
- [17] Taylor HL, Jacobs DR, Jr., Schucker B, Knudsen J, Leon AS, Debacker G. A questionnaire for the assessment of leisure time physical activities. *J Chronic Dis*. 1978;31:741-55.
- [18] Yang S, Lynch JW, Raghunathan TE, Kauhanen J, Salonen JT, Kaplan GA. Socioeconomic and psychosocial exposures across the life course and binge drinking in adulthood: population-based study. *American journal of epidemiology*. 2007;165:184-93.
- [19] Karppi J, Kurl S, Makikallio TH, Ronkainen K, Laukkanen JA. Serum beta-carotene concentrations and the risk of congestive heart failure in men: A population-based study. *Int J Cardiol*. 2013;168:1841-6.
- [20] Kunutsor SK, Khan H, Laukkanen JA. gamma-Glutamyltransferase and Risk of Sudden Cardiac Death in Middle-Aged Finnish Men: A New Prospective Cohort Study. *Journal of the American Heart Association*. 2016;5.
- [21] Kunutsor SK, Kurl S, Zaccardi F, Laukkanen JA. Baseline and long-term fibrinogen levels and risk of sudden cardiac death: A new prospective study and meta-analysis. *Atherosclerosis*. 2016;245:171-80.
- [22] Therneau TM, Grambsch PM. *Modeling Survival Data: Extending the Cox Model*. New York: Springer; 2000.
- [23] Kunutsor SK, Bakker SJ, James RW, Dullaart RP. Serum paraoxonase-1 activity and risk of incident cardiovascular disease: The PREVEND study and meta-analysis of prospective population studies. *Atherosclerosis*. 2015;245:143-54.
- [24] Kunutsor SK, Bakker SJ, Kootstra-Ros JE, Gansevoort RT, Dullaart RP. Circulating gamma glutamyltransferase and prediction of cardiovascular disease. *Atherosclerosis*. 2014;238:356-64.
- [25] Harrell FE, Jr., Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med*. 1996;15:361-87.
- [26] Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med*. 2008;27:157-72; discussion 207-12.
- [27] Pencina MJ, D'Agostino RB, Sr., Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Statistics in Medicine*. 2011;30:11-21.

- [28] Faselis C, Doulas M, Panagiotakos D, Kheirbek R, Korshak L, Manolis A, et al. Body mass index, exercise capacity, and mortality risk in male veterans with hypertension. *Am J Hypertens*. 2012;25:444-50.
- [29] Kokkinos P, Doulas M, Myers J, Faselis C, Manolis A, Pittaras A, et al. A graded association of exercise capacity and all-cause mortality in males with high-normal blood pressure. *Blood Press*. 2009;18:261-7.
- [30] Tran ZV, Weltman A, Glass GV, Mood DP. The effects of exercise on blood lipids and lipoproteins: a meta-analysis of studies. *Med Sci Sports Exerc*. 1983;15:393-402.
- [31] deFilippi CR, de Lemos JA, Tkaczuk AT, Christenson RH, Carnethon MR, Siscovick DS, et al. Physical activity, change in biomarkers of myocardial stress and injury, and subsequent heart failure risk in older adults. *J Am Coll Cardiol*. 2012;60:2539-47.
- [32] Leaf DA. The effect of physical exercise on reverse cholesterol transport. *Metabolism*. 2003;52:950-7.
- [33] Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. *Epidemiology*. 2002;13:561-8.
- [34] Church TS, Barlow CE, Earnest CP, Kampert JB, Priest EL, Blair SN. Associations between cardiorespiratory fitness and C-reactive protein in men. *Arterioscler Thromb Vasc Biol*. 2002;22:1869-76.
- [35] Sakurai T, Ogasawara J, Kizaki T, Sato S, Ishibashi Y, Takahashi M, et al. The effects of exercise training on obesity-induced dysregulated expression of adipokines in white adipose tissue. *Int J Endocrinol*. 2013;2013:801743.
- [36] Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S, et al. Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N Engl J Med*. 2000;342:454-60.
- [37] Niebauer J, Hambrecht R, Velich T, Hauer K, Marburger C, Kalberer B, et al. Attenuated progression of coronary artery disease after 6 years of multifactorial risk intervention: role of physical exercise. *Circulation*. 1997;96:2534-41.
- [38] Tulppo MP, Makikallio TH, Seppanen T, Laukkanen RT, Huikuri HV. Vagal modulation of heart rate during exercise: effects of age and physical fitness. *Am J Physiol*. 1998;274:H424-9.
- [39] Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med*. 2002;346:793-801.
- [40] Lin X, Zhang X, Guo J, Roberts CK, McKenzie S, Wu WC, et al. Effects of Exercise Training on Cardiorespiratory Fitness and Biomarkers of Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Journal of the American Heart Association*. 2015;4:pii: e002014.
- [41] American Thoracic S, American College of Chest P. ATS/ACCP Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med*. 2003;167:211-77.

- [42] Meyer T, Lucia A, Earnest CP, Kindermann W. A conceptual framework for performance diagnosis and training prescription from submaximal gas exchange parameters--theory and application. *Int J Sports Med.* 2005;26 Suppl 1:S38-48.
- [43] Goodman LS, McKenzie DC, Taunton JE, Walters MB. Ventilatory threshold and training heart rate in exercising cardiac patients. *Can J Sport Sci.* 1988;13:220-4.
- [44] Schoenhagen P, Tuzcu EM, Apperson-Hansen C, Wang C, Wolski K, Lin S, et al. Determinants of arterial wall remodeling during lipid-lowering therapy: serial intravascular ultrasound observations from the Reversal of Atherosclerosis with Aggressive Lipid Lowering Therapy (REVERSAL) trial. *Circulation.* 2006;113:2826-34.
- [45] Boyne P, Reisman D, Brian M, Barney B, Franke A, Carl D, et al. Ventilatory threshold may be a more specific measure of aerobic capacity than peak oxygen consumption rate in persons with stroke. *Top Stroke Rehabil.* 2016:1-9.
- [46] Corra U, Mezzani A, Bosimini E, Giannuzzi P. Cardiopulmonary exercise testing and prognosis in chronic heart failure: a prognosticating algorithm for the individual patient. *Chest.* 2004;126:942-50.
- [47] Ghosh AK. Anaerobic threshold: its concept and role in endurance sport. *Malays J Med Sci.* 2004;11:24-36.
- [48] Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol (1985).* 1986;60:2020-7.
- [49] Khan H, Kunutsor S, Rauramaa R, Savonen K, Kalogeropoulos AP, Georgiopoulou VV, et al. Cardiorespiratory fitness and risk of heart failure: a population-based follow-up study. *Eur J Heart Fail.* 2014;16:180-8.

Figure Title and Legend

Figure 1 Hazard ratios for sudden cardiac death, fatal coronary heart disease, fatal cardiovascular disease, and all-cause mortality by quartiles of VO_2 at VT



Hazard ratios were adjusted for age, body mass index, systolic blood pressure, high-density lipoprotein cholesterol, smoking status, alcohol consumption, prevalent coronary heart disease, history of diabetes mellitus, resting heart rate, physical activity, and socioeconomic status; VO_2 , oxygen uptake; VT, ventilatory threshold

Table 1. Baseline participant characteristics and correlates of VO₂ at VT

	Mean (SD), median (IQR), or %	Pearson correlation r (95% CI)†	Percentage difference (95% CI) in values of VO ₂ at VT per 1 SD higher or compared to reference category of correlate‡
VO ₂ at VT (ml/kg/min)	23.72 (6.10)	-	-
Metabolic equivalent	6.78 (1.74)	-	-
Questionnaire/Prevalent conditions			
Age at survey (years)	52.2 (5.4)	-0.32 (-0.36, -0.27)***	-85% (-89, -81)***
Alcohol consumption (g/week)	74.2 (113.7)	-0.09 (-0.13, -0.04)*	-40% (-54, -20)**
History of diabetes			
No	97.0	-	Ref
Yes	3.0	-	-96% (-99, -85)***
Smoking status			
Other	68.9	-	Ref
Current	31.1	-	-82% (-90, -67)***
History of hypertension			
No	70.8	-	Ref
Yes	29.2	-	-94% (-97, -88)***
History of CHD			
No	80.2	-	Ref
Yes	19.8	-	-98% (-99, -96)***
Use of anti-hypertensives			
No	81.9	-	Ref
Yes	18.1	-	-99% (-100, -98)***
Medication for dyslipidemia			
No	99.6	-	Ref
Yes	0.4	-	-100% (-100, -93)*
Physical measurements			
BMI (kg/m ²)	26.9 (3.4)	-0.36 (-0.40, -0.31)***	-87% (-90, -84)***
SBP (mmHg)	134 (16)	-0.09 (-0.14, -0.04)***	-42% (-56, -23)**
DBP (mmHg)	89 (10)	-0.13 (-0.18, -0.08)***	-53% (-64, -38)***
Physical activity (kj/day)	1,515 (1,322)	0.11 (0.06, 0.16)**	87% (41, 147)***
Peak VO ₂ (ml/kg/min)	31.4 (7.5)	0.90 (0.89, 0.91)***	26,408% (23,107, 30,178)***
RHR (beats/min)	62.5 (10.7)	-0.20 (-0.25, -0.16)***	-69% (-77, -59)***
Lipid markers			
Total cholesterol (mmol/l)	5.87 (1.06)	-0.06 (-0.10, -0.01)*	-28% (-45, -4)*
HDL-C (mmol/l)	1.28 (0.29)	0.30 (0.25, 0.34)***	458% (327, 629)***
Triglycerides (mmol/l)	1.10 (0.79-1.55)	-0.27 (-0.32, -0.23)***	-79% (-84, -73)***
Metabolic and renal markers			
Fasting plasma glucose (mmol/l)	5.32 (1.20)	-0.17 (-0.22, -0.13)***	-63% (-72, -51)***
Serum creatinine (μmol/l)	89.3 (13.7)	0.04 (-0.01, 0.09)	27% (-5, 71)
Estimated GFR (ml/min/1.73 m ²)	87.6 (18.0)	-0.05 (-0.10, 0.00)	-26% (-45, 0)

BMI, body mass index; CHD, coronary heart disease; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; RHR, resting heart rate; SD, standard deviation; SBP, systolic blood pressure; VO₂ at VT, oxygen uptake at ventilatory threshold; asterisks indicate the level of statistical significance: *, p<0.05; **, p<0.01; ***, p<0.001; †Pearson correlation coefficients between VO₂ at VT and the row variables; ‡Percentage change in values of VO₂ at VT per 1-SD increase in the row variable (or for categorical variables, the percentage difference in mean values of VO₂ at VT for the category versus the reference) adjusted for age

1 metabolic equivalent corresponds to an oxygen uptake of 3.5 ml/kg/min at ventilatory thresholds

Table 2. Associations of VO₂ at VT with sudden cardiac death, fatal coronary heart disease, fatal cardiovascular disease, and all-cause mortality

Models Oxygen uptake at ventilatory threshold	Sudden cardiac death		Fatal coronary heart disease		Fatal cardiovascular disease		All-cause mortality	
	121 cases		202 cases		312 cases		703 cases	
	Hazard ratio (95% CI)	<i>P</i> -value	Hazard ratio (95% CI)	<i>P</i> -value	Hazard ratio (95% CI)	<i>P</i> -value	Hazard ratio (95% CI)	<i>P</i> -value
Age-adjusted								
Per unit (ml/kg/min)	0.90 (0.87-0.93)	< 0.001	0.89 (0.87-0.92)	< 0.001	0.92 (0.90-0.94)	< 0.001	0.94 (0.93-0.95)	< 0.001
Per 1 MET	0.69 (0.60-0.78)	< 0.001	0.67 (0.61-0.75)	< 0.001	0.73 (0.68-0.79)	< 0.001	0.80 (0.76-0.84)	< 0.001
Quartile 1 (6.90-19.46)	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Quartile 2 (19.47-23.21)	0.72 (0.47-1.10)	0.124	0.63 (0.45-0.88)	0.007	0.67 (0.51-0.88)	0.004	0.64 (0.53-0.78)	< 0.001
Quartile 3 (23.22-27.69)	0.40 (0.24-0.67)	< 0.001	0.45 (0.31-0.66)	< 0.001	0.48 (0.35-0.65)	< 0.001	0.58 (0.47-0.70)	< 0.001
Quartile 4 (27.70-52.21)	0.18 (0.09-0.37)	< 0.001	0.18 (0.10-0.31)	< 0.001	0.27 (0.19-0.40)	< 0.001	0.39 (0.31-0.49)	< 0.001
Multivariate-adjusted*								
Per unit (ml/kg/min)	0.94 (0.90-0.98)	0.005	0.93 (0.90-0.96)	< 0.001	0.94 (0.92-0.97)	< 0.001	0.95 (0.94-0.97)	< 0.001
Per 1 MET	0.81 (0.70-0.94)	0.004	0.77 (0.68-0.86)	< 0.001	0.82 (0.75-0.90)	< 0.001	0.84 (0.79-0.89)	< 0.001
Quartile 1 (6.90-19.46)	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Quartile 2 (19.47-23.21)	1.01 (0.65-1.58)	0.965	0.87 (0.61-1.23)	0.429	0.86 (0.65-1.15)	0.311	0.72 (0.59-0.88)	0.001
Quartile 3 (23.22-27.69)	0.62 (0.36-1.08)	0.094	0.69 (0.46-1.03)	0.073	0.67 (0.48-0.94)	0.019	0.68 (0.54-0.84)	0.001
Quartile 4 (27.70-52.21)	0.37 (0.18-0.78)	0.009	0.32 (0.18-0.57)	< 0.001	0.45 (0.30-0.69)	< 0.001	0.50 (0.38-0.64)	< 0.001
Multivariate-adjusted* plus peak VO₂								
Per unit (ml/kg/min)	1.03 (0.96-1.12)	0.415	1.04 (0.98-1.11)	0.183	1.04 (0.99-1.10)	0.083	1.00 (0.97-1.03)	0.929
Per 1 MET	1.12 (0.85-1.47)	0.415	1.16 (0.93-1.43)	0.183	1.16 (0.98-1.38)	0.083	1.00 (0.90-1.12)	0.929
Quartile 1 (6.90-19.46)	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
Quartile 2 (19.47-23.21)	1.41 (0.84-2.37)	0.191	1.44 (0.95-2.17)	0.085	1.26 (0.91-1.76)	0.164	0.90 (0.72-1.13)	0.363
Quartile 3 (23.22-27.69)	1.12 (0.55-2.29)	0.753	1.67 (0.97-2.85)	0.064	1.33 (0.86-2.04)	0.199	1.02 (0.77-1.35)	0.880
Quartile 4 (27.70-52.21)	1.02 (0.36-2.91)	0.971	1.43 (0.63-3.25)	0.391	1.46 (0.79-2.71)	0.227	1.02 (0.69-1.51)	0.932

*, Hazard ratios are adjusted for age, body mass index, systolic blood pressure, high-density lipoprotein cholesterol, smoking status, alcohol consumption, prevalent coronary heart disease, history of diabetes mellitus, resting heart rate, physical activity, and socioeconomic status

VO₂ at VT, oxygen uptake at ventilatory threshold

1 MET is equivalent to 3.5 ml/kg/min of oxygen uptake at ventilatory thresholds